5

10

15

## **CLAIMS**

1. A method for calibrating data scanned from a molecular array, the method comprising:

selecting a molecular array that includes a set of calibrating probes that hybridize to a sufficient fraction of target molecules in sample solutions to which the molecular array is intended to be exposed to produce corresponding signal intensities upon reading of the calibrating probes proportional to the total concentration of target molecules in the sample solutions;

exposing the molecular array to a sample solution;

reading the molecular array to determine signal intensities for each feature of the molecular array;

calculating a collective calibration signal intensity from the signal intensities read from the set of calibrating features; and

calculating normalized signal intensities based on signal intensities read from features of the molecular array by applying to the signal intensities a normalization function that includes the calculated collective calibration signal.

- 2. The method of claim 1 wherein probes contained in the molecular array are oligonucleotides complementary to cDNA copies of cDNA transcripts of eukaryotic mRNA molecules and wherein the calibrating probes are poly(A) oligonucleotides.
- 3. The method of claim 1 wherein probes contained in the molecular array are oligonucleotides complementary to cRNA copies complementary to eukaryotic mRNA molecules and wherein the calibrating probes are poly(A) oligonucleotides.
- 4. The method of claim 1 wherein probes contained in the molecular array are oligonucleotides complementary to cDNA copies of cDNA transcripts of human mRNA molecules and wherein the calibrating probes are oligonucleotides

5

15

20

25

complementary to cDNA transcripts of Alu repeat sequences common to many human mRNAs.

- 5. The method of claim 1 wherein probes contained in the molecular array are oligonucleotides complementary to cDNA copies of the mRNA molecules and wherein the calibrating probes are oligonucleotides complementary to a synthetic nucleotide sequence appended to primers for reverse transcription of the mRNA molecules.
- 6. The method of claim 1 wherein probes contained in the molecular array are oligonucleotides complementary to cDNA copies of the mRNA molecules and wherein the calibrating probes are random-sequence oligonucleotides.

## 7. The method of claim 1

wherein calculating a collective calibration signal intensity from the signal intensities read from the set of calibrating features further includes calculating a set of collective calibration signal intensities by partitioning the signal intensities generated from the set of calibrating features into sets of similar calibrating signal intensities and calculating a collective signal intensity for each set, so that the sets of similar calibrating signal intensities each covers a discrete range of signal intensities and so that the discrete ranges of signal intensities span an overall range of signal intensities generated from features of the molecular array, and

wherein calculating normalized signal intensities based on signal intensities read from features of the molecular array by applying to the signal intensities a normalization function that includes the calculated collective calibration signal further includes applying to each signal intensity a normalization function that includes the calculated collective calibration signal calculated from the set of calibrating signal intensities within the discrete range of intensities in which the signal intensity generated from the feature of the molecular array is included.

5

10

20

30

## 8. The method of claim 1

wherein calculating a collective calibration signal intensity from the signal intensities read from the set of calibrating features further includes calculating the average calibration signal intensity from the signal intensities read from the set of calibrating features, and

wherein calculating normalized signal intensities based on signal intensities read from features of the molecular array by applying to the signal intensities a normalization function that includes the calculated collective calibration signal further includes dividing each signal intensity by the calculated average calibration signal intensity.

## 9. The method of claim 1

wherein calculating a collective calibration signal intensity from the signal intensities read from the set of calibrating features further includes calculating the mean calibration signal intensity from the signal intensities read from the set of calibrating features, and

wherein calculating normalized signal intensities based on signal intensities read from features of the molecular array by applying to the signal intensities a normalization function that includes the calculated collective calibration signal further includes dividing each signal intensity by the calculated mean calibration signal intensity.

10. A method for calibrating data scanned from a molecular array, the method comprising:

selecting a molecular array that includes features and that includes a calibration feature that includes calibrating probes that hybridize to a majority of target molecules in sample solutions, the calibration feature thereby producing a signal intensity directly proportional to the total concentration of target molecules in the sample solutions;

exposing the molecular array to a sample solution;

reading the molecular array to determine signal intensities for the features of the molecular array and for the calibrating feature; and

calculating normalized signal intensities for the features, each normalized signal intensity based on the determined signal intensity for the features and the signal intensity generated by the calibration probes.

- 11. The method of claim 10 wherein a number of calibration features are included in the molecular array, each calibration feature including calibrating probes that hybridize to a majority of target molecules in sample solutions, the calibration feature thereby producing a signal intensity directly proportional to the total concentration of target molecules in the sample solutions.
- 12. The method of claim 11 wherein a collective calibration signal intensity is calculated from signal intensities read from the number of calibration features, and wherein calculating normalized signal intensities for the features further comprises calculating normalized signal intensities based on the signal intensities read from the features of the molecular array by applying to the signal intensities a normalization function that includes the calculated collective calibration signal.
- Normalized signal intensities produced by the method of claim 12 and stored in a computer readable medium.
  - 14. Normalized signal intensities produced by the method of claim 12 and transmitted in a communications medium.

5

10

15